AD-A017 320

CLEARANCE OF BACTERIA AND ENDOTOXIN IN CONVENTIONAL OR
DECONTAMINATED MICE UNDERGOING GRAFT VERSUS HOST DISEASE
OR RADIATION-INDUCED INJURY

R. I. Walker, et al

Armed Forces Radiobiology Research Institute
Bethesda, Maryland

September 1975

Best Available Copy

DOCUMENT CONTROL DATA - R & D

Armed Forces Radiobiology Research Institute	UNCLASSIFIED
Defense Nuclear Agency	
Bethesda, Maryland 20014	N A

CLEARANCE OF BACTERIA AND ENDOTOXIN IN CONVENTIONAL OR DECONTAMINATED
MICE UNDERGOING GRAFT VERSUS HOST DISEASE OR RADIATION-INDUCED INJURY

R. I. Walker, R. J. Moon, C. B. Galley, P. F. Alm and G. D. Ledney

REPORT DATE			
September 1975		22	21

PROJECT NO. NWED QAXM	AFRRI SR75-25
Task and Subtask C 903	
Work Unit 12	

DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

	Director
	Defense Nuclear Agency
	Washington, D. C. 20305

Elimination of enteric microflora by antibiotic treatment removes a source of endogenous
infection in animals whose resistance has been compromised, but it may reduce resistance
against exogenous infection. This possibility was evaluated in conventional and decontaminated
normal mice and in those exposed to 850 rads x radiation or undergoing graft versus host dis-
ease (GVHD). When Salmonella typhimurium was perfused through mouse livers in situ ap-
proximately 70 percent of the organisms were trapped in hepatic sinusoids of normal and im-
munologically compromised animals. The trapping process was not affected by the absence of
enteric flora. However, when bacteria were injected i.v. into mice, intestinal decontamina-
tion reduced bactericidal activity in normal and irradiated mice and in those undergoing GVHD.
Approximately 50 percent of the injected S. typhimurium were killed in 20 min by conventional
animals as opposed to the 25 percent killed by decontaminated animals. Reticuloendothelial
uptake of ^{51}Cr labeled bacterial endotoxin injected i.v. was reduced in animals receiving ra-
diation or undergoing GVHD. Decontamination further compromised hepatosplenic localization
of endotoxin in the immunologically compromised mice, but not in nonirradiated animals.
Thus, oral antibiotic prophylaxis of mice, particularly those with radiation-induced injury or
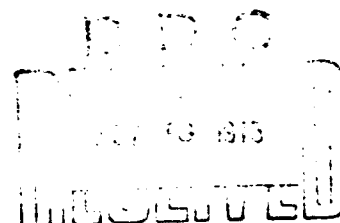those undergoing GVHD, alters clearance of bacteria and endotoxin.

i

DD FORM 1473

CLEARANCE OF BACTERIA AND ENDOTOXIN IN CONVENTIONAL OR

DECONTAMINATED MICE UNDERGOING GRAFT VERSUS HOST

DISEASE OR RADIATION-INDUCED INJURY

R. I. WALKER
R. J. MOON
C. B. GALLEY
P. F. ALM
G. D. LEDNEY

A

S. J. BAUM
Chairman
Experimental Pathology Department

LaWAYNE R. STROMBERG, MD
Colonel, USA
Director

1a

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

ACKNOWLEDGMENT

ii

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FOREWORD
(Nontechnical summary)

Almost all animals, including man, live in equilibrium with many kinds of micro-
organisms. The intestinal tract is a primary reservoir of these organisms. Although
they perform many useful services for their host, such as aiding in digestion and stim-
ulation of normal mechanisms of resistance against infection, intestinal microorga-
nisms are also a source of deadly infections when the equilibrium between them and
their hosts is upset. Thus, elimination of intestinal microorganisms by treatment with
oral antibiotics would remove a major source of endogenous infection in animals whose
normal resistance has been compromised. Removal of these microorganisms, how-
ever, may also reduce resistance against infection from outside sources. This hazard
was evaluated in the following study.

In our investigations, the normal resistance of mice was compromised by irra-
diation with ~50 rads of x rays or irradiation followed by grafting with bone marrow
from an unrelated donor resulting in graft versus host disease (GVHD). The ability of
these compromised animals to eliminate injected bacteria or endotoxin, a toxic break-
down product of some bacterial cell walls, was determined. The elimination of bac-
teria and endotoxin was also studied in immunologically compromised mice in which
intestinal microorganisms had been eliminated by oral antibiotic treatment.

When Salmonella typhimurium, a pathogenic endotoxin-containing bacteria, was
injected directly into isolated perfused mouse livers from normal and immunologically
compromised animals, approximately 70 percent of the organisms were trapped in the
network of tiny blood vessels of the liver where they could be killed. This trapping

process was not affected by the absence of intestinal microorganisms. In contrast, when bacteria were injected into the tail vein of live intact mice, the absence of intestinal organisms significantly reduced bacterial killing in normal and irradiated mice and in those undergoing GVHD. Approximately 50 percent of the injected S. typhimurium were killed in 20 minutes by animals with intestinal microorganisms as opposed to only 25 percent killing in mice with decontaminated intestines.

Mice with radiation-induced injury or undergoing GVHD could not remove endotoxin from the blood as efficiently as normal animals. Intestinal decontamination further compromised the ability of the spleen, an organ which plays an important role in removal of endotoxin from the blood, to localize injected endotoxin in immunologically compromised mice. This was not observed in normal animals.

Thus, oral antibiotic prophylaxis of mice with radiation-induced injury or undergoing GVHD impairs clearance of either bacteria or their endotoxins. Means must be sought by which host resistance to infection can be enhanced in order to compensate for the absence of intestinal microorganisms.

ABSTRACT

E..imination of enteric microflora by antibiotic treatment removes a source of endogenous infection in animals whose resistance has been compromised, but it may reduce resistance against exogenous infection. This possibility was evaluated in conventional and decontaminated normal mice and in thos · exposed to ~50 rads x radiation or undergoing graft versus host disease (GVHD). When Salmonella typhimurium was perfused through mouse livers in situ approximately 70 percent of the organisms were trapped in hepatic sinusoids of normal and immunologically compromised animals. The trapping process was not affected by the absence of enteric flora. However, when ba teria were injected i.v. into mice, intestinal decontamination reduced bactericidal activity in normal and irradiated mice and in those undergoing GVHD. Approximately 50 percent of the injected S. typhimurium were killed in 20 min by conventional animals as opposed to the 25 percent killed by decontaminated animals. Reticuloendothelial uptake of ^{51}Cr labeled bacterial endotoxin injected i.v. was reduced in animals receiving radiation or undergoing GVHD. Decontamination further compromised hepatosplenic localization of endotoxin in the immunologically compromised mice, but not in non-irradiated animals. Thus, oral antibiotic prophylaxis of mice, particularly those with radiation-induced injury or those undergoing GVHD, alters clearance of bacteria and endotoxin.

I. INTRODUCTION

Sepsis and endotoxemia contribute to mortality in animals with a severely compromised immunologic system such as that found in irradiated mice and in those undergoing graft versus host disease (GVHD). In fact, endotoxin may play a twofold role in infection. First, endotoxin from the gut pool [11,12,16,20,21] may accumulate to levels which may enhance tissue damage and alter susceptibility to endogenous or exogenous infection. Second, following establishment of infection, usually by gram-negative organisms, endotoxin shock may become a significant factor in lethality. To obviate these difficulties, numerous investigators have used oral antibiotic prophylaxis to reduce gut flora, an important source of endotoxin and/or bacterial infection. [5,14,18] However, decontamination may also alter host resistance to infection if microbial agents are accidentally or intentionally reintroduced. This hypothesis is supported by the observation that intracellular digestion is impaired in germfree animals even though phagocytic rates are normal. [3,4,10] Furthermore, in immunologically compromised animals, endotoxin or other microbial products from the gut reach host tissues. [11,12,16,20,21] Consequently, decontamination of the intestinal tract would not only block deleterious actions but might also eliminate beneficial immunological functions which may result from aseptic endotoxemia. [1,10,15]

These possible relationships between enteric flora and host resistance as a result of irradiation and GVHD led to the present investigation of bacterial and endotoxin uptake in conventional and decontaminated mice. More specifically, we wished to determine whether the reticuloendothelial system of antibiotic decontaminated animals has altered capabilities to clear challenge doses of endotoxin as well as bacterial organisms from the blood.

1

II. MATERIALS AND METHODS

Animals. All mice were obtained from Cumberland View Farms. Animals destined to undergo GVHD were male $C_{57}BL/6$ x CBA F1 ($H-2^b$ x $H-2^k$), termed B6CBF1, mice from 10 to 14 weeks of age, varying in weight from 22 to 28 g. Spleen cell donor animals were male CBA ($H-2^k$) mice 6 to 10 weeks of age. These animals were maintained in the manner previously described.[20]

X-ray exposure. Mice to be irradiated were inserted into Plexiglas restrainers and irradiated with a 300 kVp General Electric Maxitron x-ray generator operated at 20 mA. These animals were given 850 rads delivered at 40 rads/min at a skin-target distance of 90 cm. Added filtration included 2 mm Al and 1 mm Cu. All irradiations were performed between 8 and 10 a.m.

Spleen cell grafts. B6CBF1 animals destined to undergo GVHD received i.v. injections containing 5 x 10^6 allogeneic CBA spleen cells within 4 hours after irradiation.[13, 21] The manner of preparing cells for transplantation was previously described.[20]

Antibiotic decontamination of the gastrointestinal tract. Mice were placed in sterilized cages in a laminar air flow unit (100 linear ft/min), fed steam sterilized laboratory diet and given bacitracin and neomycin in acidified (pH 4) drinking water.[19, 20] Concentration of each of these antibiotics in the drinking water was 4 mg/ml. The antibiotics were provided for a 7-day period prior to irradiation and grafting and until the termination of the experiment. Mice treated with antibiotics are termed decontaminated, while mice not given antibiotics are termed conventional. The

effectiveness of decontamination was determined by cultivation of fecal pellets in thioglycollate broth.

Mouse liver perfusions. The techniques of mouse liver perfusion were similar to those of other authors.[2] Mice were injected i.p. with 200 units of sodium heparin USP diluted in saline. Anesthesia was effected within 5 to 10 min thereafter by giving an i.p. injection of 3 mg sodium pentobarbital. The portal vein and superior vena cava were cannulated, and the tube leading to the portal vein was attached to a three-way valve (Becton-Dickinson Model #MS 3033), one port of which held a syringe containing a saline suspension of bacteria to be infused while the second port was attached to a 50-ml glass syringe filled with sterile M-199. In all perfusion and clearance experiments approximately 1×10^9 viable Salmonella typhimurium strain SR-11 were used.

The liver was perfused with sterile M-199 until the effluent was visually clear of erythrocytes. The final wash was tested for sterility and any effluent having greater than 10 colony forming units (CFU) of bacteria per milliliter at this point was omitted from the final data tabulation.

Bacteria were infused in a volume of 1.0 ml followed immediately by washing with 50 ml of M-199. Preliminary studies have shown that after the wash <0.01 percent of the infused bacteria per milliliter continued to be washed out. All effluent from the perfused liver was pooled and quantitative tryptose agar pour plates were made to determine the number of viable bacteria which passed through the liver. The percent of nontrapped bacteria was calculated by the following formula:

$$\frac{\text{number of CFU recovered}}{\text{number of CFU injected}} \times 100.$$

3

The difference between the percent recovery and the total injected (100 percent) is the percent of bacteria trapped in the liver.

Microscopic examination of hepatic tissue. Occasional electron micrographs were prepared of the perfused livers to determine the location of the infused bacteria. Livers were fixed by direct infusion of 2.5 percent glutaraldehyde through one of the stopcock ports. After removal specimens were cut to the appropriate size with a razor blade and fixed for 1-2 hours at $4^{o}C$ and postfixed in 2 percent osmium tetroxide. Following dehydration in graded series of ethanol, the specimens were embedded in Araldite-Epon, sectioned with a diamond knife, stained with uranyl acetate and lead citrate, and examined with a Siemens 1A electron microscope. Unstained, thick sections were examined by phase contrast microscopy.

Clearance and killing of viable bacteria by whole animals. Mice were placed under a heat lamp for 2 to 3 min and immediately injected through the tail vein with approximately 1×10^{9} bacteria in a volume of 0.1 ml. After 20 min, mice were euthanatized by cervical dislocation and the livers and spleens removed, homogenized and the number of viable bacteria determined. The carcass minus the gastrointestinal tract, skin, feet and tail was ground in a Waring blender in 99 ml of sterile saline for 3-4 min. In preliminary studies, this procedure was found not to kill bacteria. Appropriate aliquots of all homogenates were plated for quantitation of viable bacteria. At the dilutions used, no bacteria other than the injected organisms were seen. The differences between the total bacteria recovered and the number injected were assumed to reflect the number of bacteria killed by the host in the 20 min.

4

Endotoxin. Lipopolysaccharide (Salmonella typhosa 0901 Difco, Westphal ex-
traction) was labeled with $Na_2{}^{51}CrO_4$ (New England Nuclear, specific activity 167
mCi/mg) according to the method of Braude et al.[7] Mice were inoculated i.v. with 1
mg of ^{51}Cr labeled endotoxin contained in 0.5 ml of saline. Irradiated animals and
those undergoing GVHD were tested for endotoxin clearance 2 days before death was
expected (14 and 7 days, respectively). ^{51}Cr activity was determined with the aid of
a Nuclear-Chicago Ultrascaler II well type gamma counter. All animals were euthana-
tized 1 hour after injection. Radioactive label concentrations were determined for
both lungs, both kidneys, heart, liver and spleen.

Statistics. Relative percent of the ^{51}Cr labeled endotoxin distributed among the
heart, lungs, liver, spleen and kidneys was determined. In all experimental groups
studied, 30 percent of the total injected radioactivity was found in these five organs.
A paired t-test was used to ascertain differences between treatment and control mean
values. Means were considered different only if the significance level was such that
p was less than 0.05. Data pertaining to alterations in endotoxin uptake were also
studied by analysis of variance.

III. RESULTS

^{51}Cr labeled endotoxin distribution in conventional mice. The organ distribution
of ^{51}Cr labeled endotoxin in conventional mice was determined in nonirradiated and
irradiated animals and those undergoing GVHD (Table I). Regardless of experimental
treatment, about 30 percent of the injected endotoxin was recovered in the heart, lungs,
liver, spleen and kidneys. In nonirradiated mice over 80 percent of the labeled

5

endotoxin distributed among the five organs examined was found in the liver and spleen after 1 hour. On a weight basis, splenic uptake was twice that of liver.

Table I. Organ Uptake 60 Min After ^{51}Cr Labeled Endotoxin Administration to Immunosuppressed Conventional and Decontaminated Mice

Organ examined	Experimental group*							
	Nonirradiated		Irradiated (≈50 rads) after 5 days		Irradiated (≈50 rads) after 12 days		Graft versus host disease after 5 days	
	Conventional	Decontaminated	Conventional	Decontaminated	Conventional	Decontaminated	Conventional	Decontaminated
Heart	3.1 ± 0.43	4.7 ± 2.10	3.3 ± 1.3*	8.1 ± 3.88	11.2 ± 6.24*	9.7 ± 4.41	3.5 ± 2.71	8.4 ± 3.85†
Lung	8.1 ± 1.60	11.0 ± 2.12	7.6 ± 2.71	10.0 ± 4.08	12.3 ± 0.59	13.2 ± 2.05	25.3 ± 2.4*	27.9 ± 4.84
Kidney	8.0 ± 1.35	6.1 ± 0.99	5.1 ± 1.74	10.2 ± 4.08†	11.4 ± 2.92*	14.4 ± 2.92	7.3 ± 0.90	9.6 ± 0.80†
Liver	69.8 ± 2.01	68.1 ± 4.01	77.6 ± 3.22*	67.0 ± 8.58†	56.2 ± 4.66*	58.3 ± 5.59	56.8 ± 2.71*	49.6 ± 6.40
Spleen	10.4 ± 0.66	9.9 ± 0.43	5.9 ± .51*	4.4 ± 1.76	6.1 ± 1.00†	2.5 ± 1.01†	6.7 ± 0.40*	4.3 ± 1.25†

Mice were euthanatized 60 min after endotoxin challenge and mean relative percents of the total counts per min found in the five organs examined ± standard deviation obtained. Five samples comprised each group.
* Comparison with nonirradiated conventional group showed averages were significantly different (p ≤ .05)
† Comparison with comparable conventional group showed averages were significantly different (p ≤ .05)

Five days after x irradiation hepatic uptake of labeled endotoxin was elevated by 10 percent over that observed in nonirradiated animals. A decrease of 43 percent was detected in the spleen at this time. Uptake in other organs was not altered. As shown in Table I, 12 days after irradiation both hepatic and splenic endotoxin uptake was reduced below normal levels. Liver uptake of endotoxin was reduced 28 percent below that seen at day 5 after irradiation, but splenic uptake was not decreased further at this time.

Depressed splenic and hepatic uptake of endotoxin was also found in conventional mice undergoing GVHD when compared to that seen in normal animals (Table I). Statistically greater quantities of endotoxin were detected in pulmonary tissue of animals undergoing GVHD than in lungs of normal animals. The amounts of labeled endotoxin found in the heart and kidneys were similar to those seen in nonirradiated animals.

^{51}Cr labeled endotoxin distribution in decontaminated mice. In nonirradiated animals, devoid of a demonstrable intestinal bacterial flora, of the endotoxin distributed among the five organs examined over 75 percent was again detected in the liver and the spleen (Table I). At day 5 after irradiation liver uptake of endotoxin was normal, but splenic uptake was reduced by 56 percent. Twelve days after irradiation both hepatic and splenic endotoxin uptakes were decreased when compared to those of nonirradiated animals. Uptake of labeled endotoxin in the heart and kidneys was greater in irradiated animals than in nonirradiated animals.

Comparison of ^{51}Cr endotoxin distribution between conventional and decontaminated mice. When the distribution of endotoxin was compared between conventional and decontaminated nonirradiated animals, no difference in endotoxin distribution was seen. Some statistically significant changes were noted in irradiated and GVHD mice. Increased hepatic uptake seen at 5 days after irradiation in conventional animals was not observed in decontaminated mice (Table I). In irradiated decontaminated animals, irradiated 12 days previously, splenic endotoxin uptake was 60 percent lower than that seen in irradiated conventional animals (Table I). The amount of labeled endotoxin found in the heart, lungs and kidneys was similar in decontaminated, irradiated animals and in their conventional counterparts.

In decontaminated mice undergoing GVHD, splenic uptake was below that seen in conventional animals undergoing GVHD. More endotoxin was detected in the heart and kidneys of decontaminated than of conventional animals. The amount of endotoxin removed by the livers and lungs of decontaminated mice undergoing GVHD did not differ significantly from that of their conventional counterparts.

7

Trapping of bacteria in perfused livers. To determine if hepatic removal of

bacteria from the bloodstream was altered in decontaminated animals, live bacteria

were perfused directly into the experimentally isolated mouse liver in situ. The abil-

ity of the perfused liver to trap bacteria (Table II) is not statistically different in

either nonirradiated, irradiated or GVHD affected mice, whether or not they were

decontaminated.

Table II. Trapping of Injected Bacteria by the Perfused Liver of
Conventional and Decontaminated Mice Receiving Either
No Treatment or Irradiation or Undergoing GVHD

	Percent trapping		
	Nonirradiated	Irradiated*	GVHD*
Conventional	65.5	69.7	73.2
Decontaminated	62.8	76.5	72.6

* All experiments were done 2 days before the expected time
of death for each group of mice. Irradiated mice usually
die on day 14, hence experiments were performed on days
11, 12, and 13 postirradiation; GVHD mice die on day 7
and were studied on days 4, 5, and 6.

We found that the bacteria, though trapped by the perfused organ, were still

viable. Bacteria apparently are trapped extracellularly within the sinusoids of the

perfused organ in large numbers (Figure 1). As was noted in the Methods section,

only negligible numbers of these bacteria could be removed from the liver by con-

tinued washing. The absence of opsonic components in the perfusion fluid prevented

phagocytosis of bacteria by the Kupffer cells, as long as 30 minutes after perfusion

of the microorganisms (Figure 2). In contrast, intracellular bacteria were seen only

in intact mice.
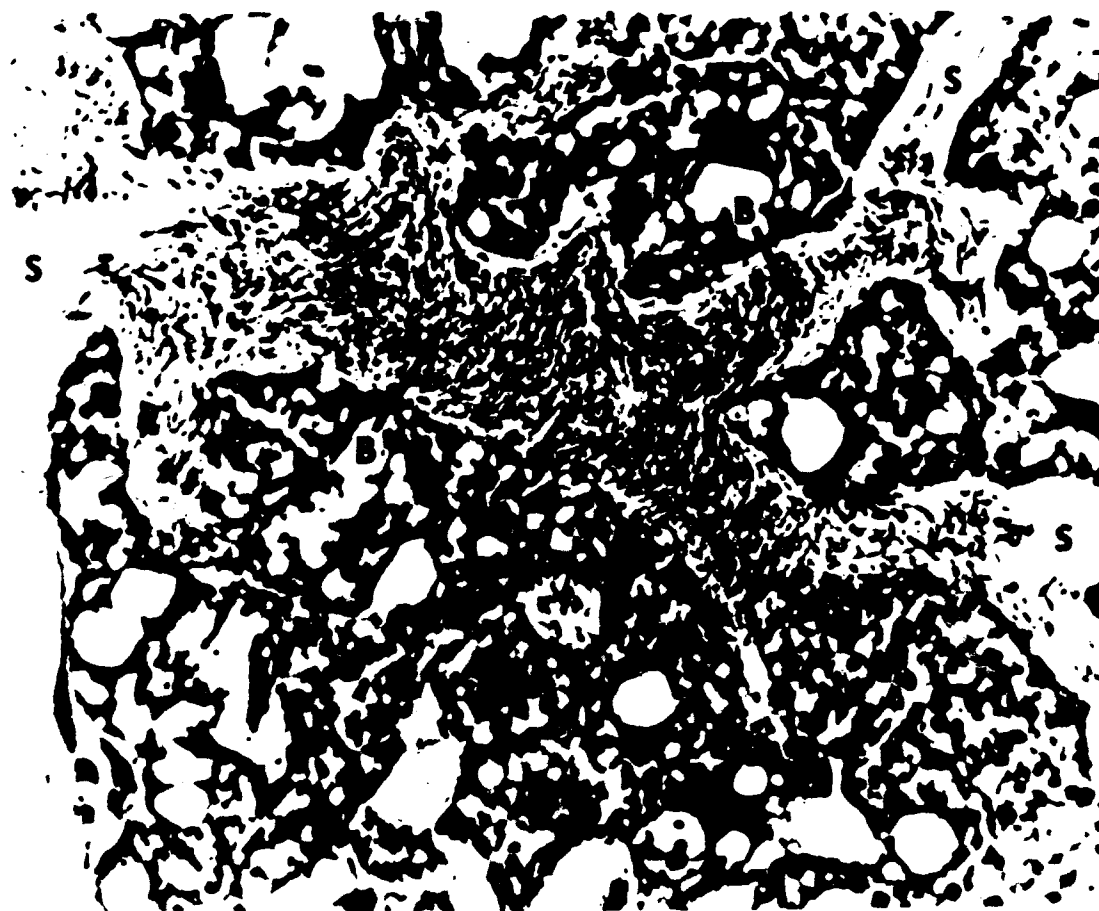
Figure 1. Phase contrast micrograph of mouse liver perfused with
1×10^9 Salmonella typhimurium. Note that organisms
(B) are trapped extracellularly in the sinusoids (S).

Clearance and killing of viable bacteria by conventional or decontaminated,

nonirradiated or irradiated mice, and by mice undergoing GVHD. Twenty minutes

after i.v. injection of 1×10^9 viable bacteria into conventional nonirradiated mice,

approximately 50 percent of the organisms were killed. No significant differences in

the percent of bacteria killed were noted if mice had been irradiated or were under-

going GVHD (Table III). Likewise, the distribution of viable S. typhimurium among

9

Figure 2. Electron micrograph of mouse liver perfused with
1×10^9 Salmonella typhimurium. Although surrounded
by bacteria (B), the Kupffer cell (K) in the liver sinus-
oid (S) appears unable to phagocytize them. X 6000.

the liver, spleen and carcass was essentially similar in all t' ee treatment groups.

When normal mice or mice with GVHD were decontaminated with antibiotics, no sig-

nificant differences were noted either in the total percent recovery or the distribution

10

Table III. Percent of Recovery of Viable Salmonella typhimurium 20 Min After Intravenous Injection of 1×10^9 Bacteria Into Both Conventional and Decontaminated, Nonirradiated, Irradiated and GVHD Mice

	Percent recovery*					
	Conventional			Decontaminated		
	Nonirradiated	Irradiated	GVHD	Nonirradiated	Irradiated	GVHD
Liver	25.5	17.6	20.7	29.2	23.3	20.6
Spleen	0.5	0.6	0.5	0.6	0.3	0.3
Carcass	25.9	23.5	29.7	38.6	59.0*	40.9*
Percent recovered	51.0	42.0	50.9	68.4	82.6	61.8
Percent killing	49.0	58.0	49.1	31.6	17.4*	38.2*

* No flora other than S. typhimurium were found in tissues at the dilutions studied
* Statistically different from appropriate control

of bacteria which remained viable within the tissues. Irradiated mice were affected more by decontamination. In this group bactericidal activity in the carcass was notably lower than in the other two groups of decontaminated animals.

We questioned whether the approximately equal recovery of viable organisms in liver and carcass reflected the true pattern of microbial distribution. However, when S. typhimurium was labeled by overnight incubation in BHI broth containing free ^{51}Cr, and then injected intravenously into mice, 39.8 percent and 45.6 percent of the injected bacteria were recovered in the liver and carcass, respectively.

Comparison of clearance and killing of viable bacteria by conventional and decontaminated mice. When decontaminated mice were compared with conventional mice, statistical differences between the two groups were detected (Table III).

11

Decontaminated mice had less ability to clear and kill bacteria than their conventional counterparts. The decrease in total killing of bacteria by decontaminated mice usually reflected enhanced numbers of S. typhimurium in the carcass. Thus, events take place in decontaminated animals which lead to decreased bactericidal capacity by these animals. Judging by these findings, we conclude that active amounts of antibiotic do not enter the bloodstream.

IV. DISCUSSION

We found that the reticuloendothelial (RE) system of mice exposed to irradiation or undergoing GVHD retains the ability to trap and kill S. typhimurium. In contrast, the RE uptake of endotoxin is reduced significantly in these animals. Deficiencies in trapping endotoxin have been shown to contribute to increased sensitivity to endotoxin.[8]

Intestinal decontamination reduces a major source of infection and endotoxin, but we found that subsequently the host becomes less efficient in clearing these agents. The mechanism responsible for the marked impairment of bactericidal activity in decontaminated animals is under investigation. No S. typhimurium were killed when suspended in whole, normal blood, although hepatic bactericidal activity was enhanced by the presence of blood (Moon, in preparation). Therefore, we feel that impairment of cellular rather than humoral bactericidal activity is induced by decontamination. This phenomenon has been reported previously in germfree mice.[4,10] Apparently continuous antigenic stimulation by intestinal microflora is required to maintain bactericidal activity in certain phagocytic cells.

Extrahepatic bactericidal systems accounted for approximately 50 percent of the bacteria killed within 20 minutes and this was reduced significantly by the

12

decontamination process. The precise location of the particular cells affected by decontamination in our study remains to be resolved. Depressed bactericidal activity was most apparent in irradiated decontaminated animals. In nonirradiated animals, normal granulocyte levels may partially mask a similar decontamination-induced depression of bactericidal activity. Likewise, in animals undergoing GVHD, grafted spleen cells may compensate partially for decreased antibacterial activity.

Intestinal decontamination did not induce dramatic alterations of endotoxin clearance. Hepatic uptake of endotoxin did not increase at 5 days after irradiation in decontaminated animals and splenic uptake of endotoxin was further depressed in decontaminated animals with radiation-induced injury or undergoing GVHD. However, only 30 percent of the total endotoxin injected was found in the organs examined (regardless of experimental treatment) so these differences are relatively insignificant. Furthermore, we have shown previously (Galley et al., in press) that decontaminated irradiated mice are actually more resistant to endotoxin challenge than conventional mice. This is probably due to the absence of intestinal endotoxin which can enter the circulation as a consequence of endotoxin shock.[20]

The exact relationships between cellular and humoral factors in normal as well as immunosuppressed animals are poorly understood. Unlike trapping of whole bacterial cells, which remains normal in immunologically compromised mice, endotoxin is not removed as efficiently from the blood. Further, although bacterial clearance remains unchanged in normal and immunosuppressed mice, immunologically compromised animals become extremely sensitive to normally sublethal (<50 µg) amounts of endotoxin (Galley et al., in press). Thus, alteration of other mechanisms such as

13

leukoc/tes and platelets,[6] plasma esterases,[17] antibodies,[8] and complement,[9] which have been implicated as cofactors necessary for endotoxin clearance, is probably responsible for increased sensitivity to endotoxin. Further work is underway to determine how factors regulating metabolism of endotoxin are affected by irradiation or GVHD.

In summary, RE organs from mice exposed to radiation or undergoing GVHD do not sequester endotoxin as efficiently as those from normal animals and this difference may contribute to increased sensitivity to this toxin. Decontamination prior to irradiation exacerbates impairment of endotoxin uptake, but probably not to a degree sufficient to affect survival. However, although bacterial clearance rema normal after radiation and during GVHD, host bactericidal activity is significantly reduced by the decontamination process. This reduction of bacterial clearance facilitates accumulation of higher levels of microorganisms in animals suffering radiation injury or undergoing GVHD.

REFERENCES

1. Arredondo, M. I. and Kampschmidt, R. F. Effect of endotoxins on phagocytic activity of the reticuloendothelial system of the rat. Proc. Soc. Exp. Biol. Med. 112:78-81, 1963.

2. Assimacopoulos-Jeannet, F., Exton, J. H. and Jeanrenaud, B. Control of gluconeogenesis and glycogenolysis in perfused livers of normal mice. Am. J. Physiol. 225:25-32, 1973.

3. Bauer, H., Horowitz, R. E., Watkins, K. C. and Popper, H. Immunologic competence and phagocytosis in germfree animals with and without stress. J. Am. Med. Assoc. 187:715-718, 1964.

4. Bauer, H., Paronetto, F., Burns, W. A. and Einheber, A. The enhancing effect of the microbial flora on macrophage function and the immune response. J. Exp. Med. 123:1013-1024, 1966.

5. Bodey, G. P., Hart, J. and Freireich, E. J. Prolonged survival of a leukemic patient in a protected environment. Am. J. Med. Sci. 256:112-121, 1968.

6. Braude, A. I. Absorption, distribution, and elimination of endotoxins and their derivatives. In: Bacterial Endotoxins, Landy, M. and Braun, W., editors, pp. 98-109. New Brunswick, New Jersey, Rutgers University Press, 1964.

7. Braude, A. I., Carey, F. J. and Zalesky, M. Studies with radioactive endotoxin. II. Correlation of physiologic effects with distribution of radioactivity in rabbits injected with lethal doses of E. coli endotoxin labelled with radioactive sodium chromate. J. Clin. Invest. 34:858-866, 1955.

8. Cuevas, P., Fine, J. and Monaco, A. P. Successful induction of increased resistance to gram-negative bacteria and to endotoxin in immunosuppressed mice. Am. J. Surg. 127:460-464, 1974.

9. Füst, G. and Fóris, G. Role of complement system in the endotoxicity-enhancing and endotoxin-detoxifying effect of serum. Med. Microbiol. Immunol. 159:141-150, 1974.

10. Gordon, H. A. and Pesti, L. The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol. Rev. 35:390-429, 1971.

11. Johnson, H. B. and Hammond, C. W. Bactericidal activity of fixed phagocytes in irradiated and unirradiated mice treated with endotoxin. Proc. Soc. Exp. Biol. Med. 130:504-508, 1969.

12. Keast, D. A simple index for the measurement of the r ating syndrome and its use in the study of the influence of the gut flora in its production. Immunology 15:237-245, 1968.

13. Ledney, G. D. Secondary disease in mice after in vitro exposure of hematopoietic cells to concanavalin A. Transplantation 14:671-682, 1972.

14. Levine, A. S., Siegel, S. E., Schreiber, A. D., Hauser, J., Preisler, H., Goldstein, I. M., Seidler, F., Simon, R., Perry, S., Bennett, J. E. and Henderson, E. S. Protected environments and prophylactic antibiotics. A prospective controlled study of their utility in the therapy of acute leukemia. N. Engl. J. Med. 288:477-483, 1973.

15. Milner, K. C., Rudbach, J. A. and Ribi, E. General characteristics. In: Microbial Toxins, Vol. IV. Bacterial Endotoxins, Weinbaum, G., Kadis, S. and Ajl, S. J., editors, pp. 1-65. New York and London, Academic Press, 1971.

16. Rosoff, C. B. The role of intestinal bacteria in the recovery from whole body radiation. J. Exp. Med. 118:935-943, 1963.

17. Skarnes, R. C. Host defense against bacterial endotoxemia: mechanism in normal animals. J. Exp. Med. 132:300-316, 1970.

18. Vossen, J. M. and van der Waay, D. Reverse isolation in bone marrow transplantation: ultra-clean room compared with laminar flow technique. I. Isolation systems. Rev. Eur. Etud. Clin. Biol. 17:457-461, 1972.

19. Waaij, D. van der and Sturm, C. A. Antibiotic decontamination of the digestive tract of mice. Technical procedures. Lab. Anim. Care 18:1-10, 1968.

20. Walker, R. I., Ledney, G. D. and Galley, C. B. Aseptic endotoxemia in radiation injury and graft-vs-host disease. Radiation Res. 62:242-249, 1975.

21. Wilson, R., Barry, T. A. and Bealmear, P. M. Evidence for a toxic substance of bacterial origin in the blood of irradiated mice. Radiation Res. 41:89-103, 1970.